

# **Gears MANIQ 1.1**

# **USER MANUAL**



Document Number

P/N 226 R3



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## 1. Installation

First, three different products need to be installed to run the MANIQ plugin for Mnova, and in the following order:

- Mnova (minimum version: 14.3.2.32716)
- Mgears (minimum version: 2.4.2.11585)
- MANIQ (minimum version: 1.1.0.11780)

Nombre	Fecha de modificación	Тіро	Tamaño
🔀 MestReNova-14.3.2-32299_x64.msi	15/02/2023 12:03	Paquete de Windows Installer	468.996 KB
🔋 Mnova-Gears-2.4.2.11834.zip	15/02/2023 12:02	Carpeta comprimida (en zip)	11.384 KB
腸 Mnova-MANIQ-1.1.0.11780.zip	15/02/2023 12:02	Carpeta comprimida (en zip)	355 KB

While Mnova has a regular installer, Mnova Gears and MANIQ need to be installed via the Mnova interface. To this end, you can just drag and drop the installers into the Mnova interface (please note that MANIQ must be installed *after* Mnova Gears).

(	👂 Install Mnova I	Plug-in		?	×			
		Mnova Gears						
	Description:	Mnova Gears - General Enterprise Automation Rapid Solution						
	Mnova Gears uses <u>qPDF version 10</u> (aPDF is licensed under Apache License 2.0)							
	Version:	2.4.2.11834						
	Release Date:	Monday, 13 February 2023						
	Requires:	MestReNova (>=14.3.1-31090)						
	Plug-in	ready to be installed.						
		Install		Can	cel			



	MANIQ	
Description:	Mnova Automatic NMR Identification and Quantification Advanced Plugin	
Version:	1.1.0.11780	
Release Date:	Wednesday, 8 February 2023	
Requires:	MestReNova (>=14.3.2-32176) Mnova Gears (>=2.4.2.11585)	
🕑 Plug-in	ready to be installed.	

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### 2.1. Configuring Mgears

Before running the MANIQ plugin, Mgears must be appropriately configured. First, open **Mgears** from the **Automation** menu in Mnova.

File O Mgears V	Home View	Molecule	Prediction	Tools	Data Analysis	Database	Verification	Elucidation	Chemometrics	Binding	Automation	Quantitati
Pag 🗞 N	Inova Gears										?	×
- <b>+</b>	Input 😵 P	rocessing	Plugins	🔀 Desi	gn 📑 Outp	ut 🔅 Se	ttings					
Real Time	Using Auto Main Directory: Optional Direct	IS	s/Usuario	ments Selec								
Filter	esume 🚺 🏚 Lo	ad Settings	Save Setting	gs 📋 I	nport Settings	<ul> <li>Export 5</li> </ul>	Settings 👻				Cancel	Run

Then, proceed to set a Main Directory in the Input tab.

% Mnova Gears	? ×
Find Processing Plugins 🔀 Design 💽 Output	
Image: Section Figure 1       Image: Section Figure 1         Image: Section Figure 1       Image: Section Figure 1 <td></td>	
Real Time	
Advanced Options	>
Filter	
Resume         Image: Load Settings         Save Settings         Import Settings         Import Settings         Cancel	Run

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The **Main Directory** folder should contain files with the NMR spectra of the samples you want to analyze. Additionally, you can add different filters to select between the **FID** and the **Spectrum** files (it is strongly recommended that you choose the latter for the calculation of absolute quantifications).

% Mnova Gears ?	×
Input 😵 Processing 🛞 Plugins 🐹 Design 📑 Output 🔅 Settings	
	ē,
Main Directory:     C:/data/manig/manual-demo/input       B     Optional Directory:	
Image: Spectrum *       13C       FID *       19F       FID *       HSQC       FID *       HMBC       FID *       FID *         Image: Spectrum *       13C       FID *       19F       FID *       HSQC       FID *       HMBC       FID *       FID *         Image: Spectrum *       13C       FID *       19F       FID *       HSQC       FID *       FID *       FID *         Image: Spectrum *       10CSY       FID *       ROESY       FID *       H2BC       FID *       COSY       FID *       FID *         Image: Change All       FID *       Image: Spectrum *       Image: Spectrum *       FID *       FID *       FID *         Image: Spectrum *       Image: Spectrum *       Image: Spectrum *       Image: Spectrum *       FID *       FID *         Image: Spectrum *       Image: Spectrum *       Image: Spectrum *       Image: Spectrum *       Image: Spectrum *       FID *         Image: Spectrum *       Image: Spectrum *       Image: Spectrum *       Image: Spectrum *       Image: Spectrum *       FID *         Image: Spectrum *       Image: Spectrum *       Image: Spectrum *       Image: Spectrum *       Image: Spectrum *       Image: Spectrum *       Image: Spectrum *       Image: Spectrum *       Image: Spectrum *	
Master File: Main SDF File: Main SDF File: Allow More than One Experiment of Each Type Filter by: Creation Date Filter by Date From: 1 Jan 1970  Filter by: Creation Date Creation Date Filter Subset Only 1000  Creation Date Cr	
Filter         Import Settings         Import Settings         Import Settings         Import Settings	Run

If you want to check that the documents you want to analyze are being correctly detected by Mgears from the **Main Directory** folder, you can use the **Automatic Detection** tool.

og Mn	ova Gears			? ×
€	Input 😵 Processing 🚱 Plugins 🐹 Design 📑 Output 🔅 Settings			
<b>Sol</b> Disk	Using Automatic Detection			
	Main Directory: C:/data/maniq/manual-demo/input			
B	Optional Directory: 🔲 💊 Experiments Found: 1	?	×	
Real Time	Image: Normal System       List of detected experiments:         Experiment: LYSINE_ALANINE       Image: List of detected experiments:         Experiment: LYSINE_ALANINE       Image: List of detected experiments:         List of detected experiments:       Image: List of detected experiments:         Experiment: LYSINE_ALANINE       Image: List of detected experiments:         List of detected experiments:       Image: List of detected experiments:         List of detected experiments:       Image: List of detected experiments:         List of detected experiments:       Image: List of detected experiments:         Advanced Options       Image: List of detected experiments:         Main SDF File:       Image: List of detected experiments:         Allow More than One       Image: List of detected experiments:	ОК		FID * FID * FID *
	Experiment Selection Mode: FID/Spectrum Filter by Date From: 1 Jan 1970 To:	1 Jan 197	70 - Fi	ilter by: Creation Date 💌
	Preview Subset Only Regular Expression to Adapt Match /			/ 👿
Filter				
R	esume 🔹 Load Settings 🔹 Save Settings 📫 Import Settings 👻 📩 Export Settings 👻			Cancel DRun

M



In the **Output** tab, it is necessary to set up the output folder in which the results will be created. It is also possible to configure whether you want Mnova and/or PDF files with the analyzed samples to be copied into the results folder, but this is not immediately necessary as the MANIQ analysis results will also be output in another file.

∞ Mnova Gears	?	×
Input 😵 Processing 🔗 Plugins 🐹 Design 💽 Output		
Disk		
Directory: C:/data/mgears-results Add Incremental Numbering 🖸 🗰	Only Nickna	ime
Mnova		
Save Mnova Document in: Save a Copy of the Mnova Document wit	h the Raw	Data
Save in Results Folder O Save with the Raw Data O Save PDF in:		
Protect PDF from Editing Add Analysis Type in the Name of the PDF Embed in Mnova Document Save PDF in a New Record Save PDF in a New Record		
Expert		
		>
PPResume Load Settings Save Settings I II Import Settings * K Export Settings *	cel 🚺	Run

Finally, you must add the MANIQ plugin to the set of plugins that will be run with Mgears. For this, go to the **Plugins** tab, find and click on the corresponding MANIQ brick in the left-hand side list, and click on **Add**. Following that, the MANIQ brick should appear on the right-hand side, which is the list of plugins that will be used in the Mgears analysis.



For more details on the Mnova Gears general configuration, please refer to the Mnova Gears manual.



#### 2.2. Configuring MANIQ

You can now proceed to set up the MANIQ plugin. In the **Plugins** tab, click on **MANIQ** and then click on **MANIQ Plugin Settings** in the middle column. This will open the **MANIQ Settings** dialog.

🗞 Mnova Ge	ears							?	$\times$
🛃 Input	Processing	Plugins	🔀 Design	Output	Settings				
SQA	IQ			×	Add Add Remove Custom Plugin Delete Custom Plugin MANIQ Plugin Settings	MANIQ			
Resume	Load Settings	Save Setting	gs 📋 Impor	t Settings 🔹 🖡	Export Settings	-		Cancel	Run

#### 2.2.1. The Compounds Library tab

The most important part of the MANIQ plugin configuration is the **Compounds Library**. This library consists of an Mnova document with the NMR spectra of the reference compounds that the user wants to include in the identification and quantification analysis. The structure of this document must be configured as follows: on each page of the document, there should be an NMR spectrum of a compound along with its corresponding molecular structure. An example of a compound library document is as follows:

# 📕 전 약 🚔 = Elle Home View Molecule Prediction Tools Data-Anabrais Database	MR Mesteriora	
Construction         Previous	Secture Toolbal     Enter Page ←       Image: Duration toolbal     Image: Duration toolbal       Image: Duration toolbal     Image: Duration toolbal	
g Compounds Ø ×	Document 1 ×     Document 1 ×	Pages 🗗 🗙 🙀
Image: Constraint of the state of		1. (1) alarine. 100.600. tr
1         Microsoft Fass:         14-511           1         Remote Market Fass:         14-511           1         Color:         X Monte Market Fass:           1         Color:         X Monte Market Fass:           1         Aniguments         None Market Fass:           1         Holectar Composition:         C-98.30% H:d5.5% N:d5.16% O-21.8%           1         DBE:         C-98.30% H:d5.5% N:d5.16% O-21.8%	illionine.100.550.1/r C.JBR-KE.Jegord/BioproductorPureCompounds/Jamine/100(pdate/709)	
000000000000000000000000000000000000	3 0 0 1 2040 3040	الله الله الله الله الله الله الله ال
NH2 stagements below	HO <sup>2</sup> 4 <sup>4</sup> 6 2 5 5 25×10 25×10	· - · · · · · · · · · · · · · · · · · ·
	20.00 3.79 1.90	
	- 1540	
	55%10	,
4		

It is also important that each molecular structure is correctly labeled, as the name assigned to each compound is the label given to the corresponding structure (to view this information, you can open the **Compounds table**: **View**  $\rightarrow$  **Tables**  $\rightarrow$  **Molecule** and then check **Compounds** and click **OK**; you can then review the **Label** field for each compound).



When the compounds library is ready, select it from the **MANIQ Settings** dialog. A **table** will appear with the information for each compound taken from the document.







Compounds Library Ana C:/data/maniq/manual-dem C:/data/manual-dem C:/data/manual-de	nalysis Reports mo/library.mnova Name HMDB0001310 (D-Alanine)	nH	🖆	0	Compor	unds Lib	xary A	nalysis Reports			0
C:/data/maniq/manual-dem	mo/library.mnova Name HMDB0001310 (D-Alanine)	nH									_
Sel.         1H Page           Image         Image           Image </td <td>Name HMDB0001310 (D-Alanine)</td> <td>nH</td> <td></td> <td colspan="7">C:/data/maniq/manual-demo/library.mnova</td> <td>1</td>	Name HMDB0001310 (D-Alanine)	nH		C:/data/maniq/manual-demo/library.mnova							1
	HMDB0001310 (D-Alanine)		Mol. Mass (g/mol)			Sel.	1H Page	Name	nH	Mol. Mass (g/mol)	1
<ul><li><b>₹</b></li><li><b>₹</b></li></ul>		4	89.09			$\checkmark$	1	alanine.100.600.1r	4	89.09	
	HMDB0000182 (L-Lysine)	9	146.19		Q	$\checkmark$	2	lysine.100.1.1r	9	146.19	
					G G						

**Important.** When opening a library for the first time, Mnova will show the number of observable protons (nH) for each molecule, as calculated according to a set of rules. However, it is advised that the user reviews these values the first time the library is loaded as they are critical to a correct quantification. If required, the nH values (but also the Molecular mass and Name fields information) can be manually edited by clicking on each field and typing the new values. After editing, the user must click on the **Save** button.





Note that it is possible to use anything from the entire compound library to a particular selection of compounds for the analyses. The user can easily check/uncheck the compound rows to include/exclude them from the analysis, respectively.

It is also possible to run an analysis of the reference library to detect compounds that can potentially be falsely identified as components of certain samples by the Direct Peak Search algorithm (further information about this algorithm can be found in the *Analysis tab* section). The analysis will generate a list with **Expected False Positives**, **Possible False Positives**, and **Unlikely False Positives**, as shown in the image below.



#### 2.2.2. The Analysis tab

The Analysis tab is divided into three different sections: Identification, Quantification, and Exclusion Regions.

In the **Identification** section, there are two preconfigured modes: **Compound ID** and **Spectrum ID**. Compound ID is for mixtures of samples in which the compound signals may be overlapped with those of other compounds. Spectrum ID, by contrast, is for samples in which only one compound is present. Although it is advised that one of these two settings be used in the general case, an additional mode called **Custom** enables the user to select which method(s) they want to use.



To date, there are four different identification methods, which can be used separately or jointly:

- **DPS (Direct Peak Search)**. This method matches all the peaks for each compound with the corresponding peaks in the sample spectrum. If one or more peaks cannot be matched, then it discards the compound. This is the recommended method for use with mixture samples.
- **Cosine Similarity**. This method uses cosine similarity to compare the compounds' spectra with the sample spectrum, otherwise discarding compounds according to a threshold set by the user. Additionally, the user can enable Cross Correlation or Align Multiplet Regions Separately to improve the results in the presence of shift changes.
- **Pearson Correlation Coefficient**. This method uses the Pearson correlation coefficient to compare the compounds' spectra with the sample spectrum, discarding compounds according to a threshold set by the user.
- **13C Analysis\***. This option uses Mnova Verify to compare the 13C spectra associated with the compounds with the 13C spectrum of the sample (if any). It enables an additional column in the compounds' library table. If no 13C spectrum is found for a compound, it will use the 13C prediction from the corresponding molecule instead. (\*Using this method requires valid licenses for Mnova NMR Predict and Mnova Verify plugins.)

In the **Quantification** settings, there are two modes: **Default** and **Custom**. The first works in most situations, and uses the **f Factor Method**, an analysis algorithm internally developed by Mestrelab. The second allows the user to select which method they want to use, and in the case of the **Integral Transforms Method**, there are different settings to choose from. The relative concentrations reported in the results can be shown in g % or mol %.

Mode: Custom		•	Mode:	Default
Quantification		<b>\$</b>	Integral Transforms	Method
Jnits: ✓ Absolute Quantifica ○ Internal Calibrant Units: Decimal Places:	mol %	ULCON)	Integral Transforms T Maximum Order: Use Model Select AIC (Akaike Info MDL (Minimum D	ype: Euclidean Distance 20 tion Criterion ormation Criterion) Description Length)
dusion Regions	Max (ppm)	+ -		OK Cancel Reset

You can enable the **Absolute Quantification** — the units and the decimal places for the returned values can be configured— but some information needs to be provided for each analysis mode:

- In the **External Calibrant** mode, the "eretic" file provided by TopSpin must be available inside the sample spectrum directory.
- In the Internal Calibrant mode, a reference compound must be selected in the Compounds Library table (the Reference column is enabled once this mode is selected). Its actual concentration must be specified in the comment field of the sample spectrum, as shown below (this value must be in mmol/l).



On the lower part of the **Analysis** tab, there is a section where **Exclusion Regions** can be added to the MANIQ analysis. As their name suggests, those regions will be completely excluded from the identification and quantification analyses.

<b>0</b> 0 MA	NIQ Settings	?	×							
	ی 🖬									
Com	pounds Library Analysis Reports									
V I	✓ Identification									
м	ode: Compound ID	¢								
⊻ (	2 🍫 Define Exclusion Region ? 🛛 🗙	٦.	-							
M	Minimum: 2.3000 ppm 🌻	•								
	Maximum: 2.7000 ppm 🗘	N)								
	OK Cancel	•								
I		¢								
Excl	usion Regions	K	_							
	Min (ppm) Max (ppm)									
	-0.1 0.1	-								
		1								
	ОК	Canc	el							

#### 2.2.3. The Reports tab

In the **Reports** tab you can find additional settings, all of which are self-explanatory. You can also export the default layout template file for using it as basis for your own one. More information about these settings is available in the corresponding tooltips.

% MANIQ Settings ? X									
8									
Compounds Library Analysis Reports									
Result Spectrum									
Title Format: Test Sample: %SAMPLENAME									
$\checkmark$ Stack Detected Compounds with Test Sample Spectrum									
Show Stack as Superimposed									
✓ Use Smart Zoom									
✓ Restore Cuts from Excluded Regions									
Identification									
Show Cosine Similarity									
Show Pearson Correlation Coefficient									
Show 13C Analysis									
Export									
<ul> <li>Export Results to JSON</li> </ul>									
✓ Export Results to CSV									
Copy Compounds Library to the Results Folder									
Layout Template									
Use Default Layout Template									
<ul> <li>Use Custom Layout Template</li> </ul>									
✓ Multiple Page Layout Template									
OK Cancel									



# 3. Running the MANIQ analysis

#### 3.1. Running Mgears

After Mgears and MANIQ have both been set up correctly, you are now in position to run the analysis. To do this, just click on the **Run** button in the main Mgears dialog.

Input Processing Plugins 🔀 De	📑 Output 🛛 🧔 Settings	
SQA MANIQ	Add Add Remove Maniq Delete Custom Plugin Delete Custom Plugin Custom Settings	

A new dialog will then be shown in which the user can watch the progress of the analysis file by file.

Mnova Gears	
Log:	
[2022-10-10T08. 17.25] Running Mgears 2.4. 1. 10852	
	-
Estimated Time Left: Calquiating	
Estimated hime bene ouropaiding hi	



After Mgears has finished the analysis, a new folder will appear in the Output directory configured in the Mgears settings, named after the date and time of the analysis.

Windows (C:) > data > mgears-results							
Nombre	Fecha de modificación	Тіро	Tamaño				
🔒 2022-10-10T08.17.24_MANIQ-Analysis-1	10/10/2022 8:17	Carpeta de archivos					

Inside this folder, there are Mnova documents and PDF files containing the analyzed mixture spectra (assuming the user checked the appropriate options in the **Output** tab). The actual results of the MANIQ analysis can be found in the MANIQResults.html file.

Nombre	Fecha de modificación	Тіро	Tamaño
css	10/10/2022 8:21	Carpeta de archivos	
📊 data	10/10/2022 8:21	Carpeta de archivos	
documents	10/10/2022 8:21	Carpeta de archivos	
📙 images	10/10/2022 8:21	Carpeta de archivos	
🔜 js	10/10/2022 8:21	Carpeta de archivos	
📙 pdf	10/10/2022 8:21	Carpeta de archivos	
📔 2022-10-10T08.21.12.log	10/10/2022 8:21	Archivo LOG	1 KB
2022-10-10T08.21.12.resume	10/10/2022 8:21	Archivo RESUME	1 KB
MANIQResults.csv	10/10/2022 8:21	Hoja de cálculo d	1 KB
MANIQResults.html	10/10/2022 8:21	Firefox HTML Doc	6 KB
📔 MANIQResults.json	10/10/2022 8:21	Archivo JSON	3 KB
🥁 settings.mgrs	10/10/2022 8:21	Archivo MGRS	8 KB

This HTML file includes a table. The rows represent the mixture files, and the columns represent the reference compounds in the Compounds Library. The results of the MANIQ analysis are shown within the table, where green indicates that the compound was detected in the mixture, and white otherwise. The values and results for each identification method used are shown, along with the relative quantification results. If you enable the Absolute Quantification, the absolute concentrations will also be shown here.

MANIQ Results						
Parameters						
Parameter	Value					
Results Directory	C:/data/mgears-results/2022-10-10T08.26.49_MANIQ-Analysis-3					
Started On	2022-10-10T08:26:49					
Completed On	2022-10-10T06:26:57					

#### **Detailed Results**

Show All	✓ entries		Copy CSV	Columns	PDF	Print			Search:
#	Name	Туре		≑ H	MDB0001	310 (D-Alanine)		+ HMDB0000182 (L-Lysine	)
1	LYSINE_ALANINE	DPS (Score)							
2	LYSINE_ALANINE	Identification (Result)					Yes		Yes
3	LYSINE_ALANINE	Identification (Score)					0.75		1.00
4	LYSINE_ALANINE	Rel. Conc. (%)							6.90
5	LYSINE_ALANINE	Abs. Conc. (g/l)					7.64		0.57
Obsuring 4.4	a E of E optring								

howing 1 to 5 of 5 entries

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Next

Alternatively, you can view the results from the **Mgears Viewer** tool that you can launch from the **Automation** tab in the Mnova ribbon. To add the results to the viewer, you must press the **Load Data** button, and choose the output folder.

The results will be loaded and can be reviewed on a per sample basis. The **Well Plate** will show each sample in green if at least one compound was identified, and in red otherwise. In the **Results** section, several tabs will show the different partial results for each sample: Identification, Quantification, Cosine Similarity, etc.



For more details on the Mnova Gears options, please refer to the Mnova Gears manual.